Aliso: A Journal of Systematic and Evolutionary Botany

Volume 22 | Issue 1 Article 13

2006

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Aliso 22, pp. 159–171

PATTERNS OF FLORAL STRUCTURE AND ORIENTATION IN *JAPONOLIRION, NARTHECIUM,* AND *TOFIELDIA*

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ABSTRACT

Floral evolution requires reassessment in basal monocots, including species formerly assigned to Melanthiaceae, in the light of recent developments in the molecular phylogenetics of monocots. We have investigated flowers of *Tofieldia* (Tofieldiaceae), *Japonolirion* (Petrosaviaceae), and *Narthecium* (Nartheciaceae). We confirm Engler's (1888) hypothesis that orientation of lateral flowers in monocots is dependent on presence and position of additional phyllomes on the pedicel. The type of floral orientation that occurs in *Tofieldia* is unusual for monocots, since the additional phyllomes are represented by calyculus scales rather than a bracteole, and the outer whorl tepals are initiated alternating with the calyculus scales. In *Japonolirion* and *Narthecium,* a bracteole is inserted in an adaxial-transverse or transverse position; either the outer median tepal is adaxial or no single tepal is inserted in the median position. In *Tofieldia,* the pedicel has a calyculus of an abaxial and two adaxial-transverse phyllomes; the outer median tepal is adaxial. Additional phyllomes on the pedicel are not adaxial, in contrast to adaxial prophylls in the vegetative regions. The presence or absence of a bracteole or calyculus is taxonomically important. *Tofieldia pusilla* differs from the other species of *Tofieldia* examined in the absence of a flower-subtending bract, but the calyculus demonstrates some bract-like features in position, structure and development, which can be interpreted as a hybridization of developmental pathways. The abaxial calyculus scale of *T. coccinea* is delayed in development.

Key words: bract, development, flower, *Japonolirion, Melanthiaceae,* morphology, *Narthecium, Tofieldia.*

INTRODUCTION

Prior to recent taxonomic rearrangements based partly on molecular data (e.g., Angiosperm Phylogeny Group [APG] 1998), genera that were formerly ascribed to Melanthiaceae s.l. (or Liliaceae–Melanthioideae) were considered to represent some of the most basal monocots because of their relatively "primitive" flowers with mostly free floral parts and at least partially apocarpous ovaries (Melchior 1964; Dahlgren et al. 1985; Takhtajan 1987, 1997; Thorne 1992). Therefore, they were grouped partly on the basis of putatively plesiomorphic characters and partly because of their geographical distribution and habitat preferences, as they are mostly marsh-loving northern temperate herbs.

Several recent studies, both molecular and non-molecular (e.g., Ambrose 1980; Chase et al. 1995; Stevenson and Loconte 1995) have demonstrated that Melanthiaceae s.l. are polyphyletic. Takhtajan (1994, 1997) proposed splitting Melanthiaceae into several separate but closely related families. Analyses of molecular data (e.g., Chase et al. 1995) have at least partly confirmed segregation into separate families, but placed them in different orders (APG 1998, 2003). Thus, the genera that were formerly placed in Melanthiaceae are now dispersed in four families: Nartheciaceae (Dioscoreales), Tofieldiaceae (Alismatales), Petrosaviaceae (Petrosaviales) and Melanthiaceae s.s. (Liliales), the latter including several tribes. The position of Petrosaviaceae (*Japonolirion* Nakai and *Petrosavia* Becc.) remains unresolved; in most recent analyses they are placed in an isolated position as sister to all monocots except *Acorus* L. and Alismatales (e.g., Cameron et al. 2003; Chase 2003).

Floral evolution in basal monocots requires reassessment in the light of new phylogenetic data. Buzgo and Endress (2000), Buzgo (2001), and Buzgo et al. (2006) compared in detail the floral morphology of some basal monocot groups such as Acoraceae (Acorales) and Araceae (Alismatales), and demonstrated the taxonomic importance of the pattern of floral orientation and presence/absence of a bract. Here we investigate these features in some former Melanthiaceae s.l., especially in the relatively basal monocot *Tofieldia* Huds. (Tofieldiaceae), which possesses unusual inflorescence structure (Remizova and Sokoloff 2003). The rare Japanese species *Japonolirion osense* (Petrosaviaceae) is relatively poorly known and requires further examination since several authors have noted its similarity to *Tofieldia* (Tamura 1998; Cameron et al. 2003). Furthermore, both Nartheciaceae and Petrosaviaceae occupy relatively pivotal phylogenetic positions close to the "spine" of the monocot tree (e.g., Chase et al. 2000; Chase 2003). Many aspects of floral morphology, anatomy, and vasculature have been extensively studied in Melanthiaceae s.l. (El-Hamidi 1952; Leinfellner 1963; Daumann 1970; Eie 1972; Utech 1978*a*, *b*, *c*, *d*, 1984; Sterling 1979; Utech and Kawano 1981; van Heel 1988; Cruden 1991; Zomlefer 1997; Tamura 1998; Igersheim et al. 2001; Rudall 2002). However, relatively few of these investigations have discussed bracts, bracteoles, and floral orientation and initiation (e.g., Eichler 1875; Engler 1888; Endress 1995).

MATERIALS AND METHODS

Morphology and development of inflorescence and flower were examined in three species of *Tofieldia* (Tofieldiaceae,

Table 1. Collection data of material examined (in addition to material listed in Remizova and Sokoloff 2003).

Species	Location	Date	Collector
Japonolirion osense Nakai	UK, Cultivated material from RBG Kew (1996–2718)	n/a	n/a
Narthecium ossifragum (L.) Huds.	UK, Ranmore Common	1998	C. A. Furness, P. J. Rudall
Tofieldia coccinea Richards	N Russia, Taymyr, near Novaya River 74.5°N, 107.7°E	16.7.2002, 26.7.2002, 6.8.2002	E. B. Pospelova, I. N. Pospelov
	E Russia, Commander Islands, Medny Island	14.8.2002, 15.8.2002	E. G. Ivashkin, N. A. Bocharova
T. okuboi Makino	Japan, Honshu Island	27.9.2002	P. Yu. Zhmylev, K. Kondo
T. pusilla (Michx.) Pers.	NW Russia, Karelia, White Sea Biological Station of Moscow University	17.7.2002, 28.7.2002	M. V. Remizowa, E. N. Bubnova

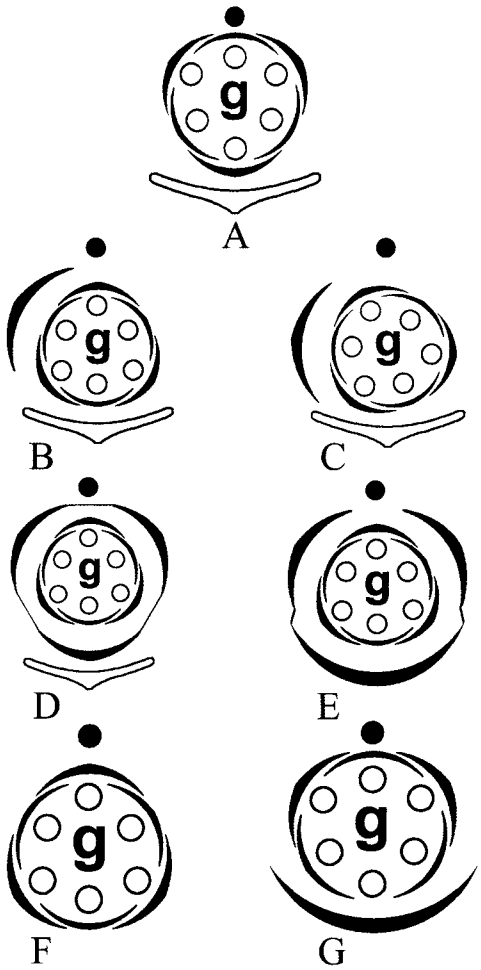

Fig. 1.—Simplified floral diagrams (modified from Remizova and Sokoloff 2003). A, *Veratrum* and most lilioid monocots (Type 1 of flower orientation in former Melanthiaceae); B and C, *Japonolirion osense* and *Narthecium ossifragum* (Type 2 of flower orientation in former Melanthiaceae); D, *Tofieldia* sect. *Tofieldia* (Type 3 of flower orientation in former Melanthiaceae); E, *Tofieldia* sect. *Unibracteatae* (Type 3); F, members of Araceae with trimerous perfect flowers (after Buzgo 2001); G, *Acorus* (after Buzgo and Endress 2000). Black circle = inflorescence axis; white circle = stamen; g = gynoecium; black arc = tepal, scale of calyculus and bracteole; white arc = subtending bract; gray arc = organs of "hybrid" nature.

Alismatales), monotypic *Japonolirion* (Petrosaviaceae, Petrosaviales), and one species of *Narthecium* Huds. (Nartheciaceae, Dioscoreales) (Fig. 1). Details of material examined are given in Table 1. Species of *Tofieldia* examined are in two sections: sect. *Tofieldia* (*T. coccinea* and *T. okuboi*) and sect. *Unibracteatae* Leonova (*T. pusilla*).

Plant material was fixed in FAA or 70% ethanol and stored in 70% ethanol. For scanning electron microscopy (SEM), parts of inflorescences and flowers were dehydrated in 100% ethanol and 100% acetone. Dehydrated material was critical point dried and sputter-coated with Pt/Pd.

RESULTS

Narthecium ossifragum *(Nartheciaceae)*

Inflorescences are racemose. Flowers are trimerous, actinomorphic, pedicellate, and subtended by a well-developed bract and have a single bracteole; the bract is slightly longer and broader than the bracteole (Fig. 2, 3). There are six tepals in two whorls of three, free at early stages of development and united in a floral tube at later stages (Fig. 4).

At early stages of flower development, the bract, bracteole, and tepals have secretory hairs on their tips (Fig. 2), but these are less prominent at later developmental stages (Fig. 3). The secretion products of these hairs hold the tepals together, probably to protect developing stamens and carpels.

Floral orientation in *Narthecium ossifragum* is variable due to the unstable position of the bracteole. The diagrams in Fig. 1 illustrate two limits of variation of bracteole orientation; both types and intermediate forms may be present in the same inflorescence. Bracteoles may be inserted to the left or the right of the flower. In some cases flowers possess the outer median tepal in adaxial position; the bracteole occurs opposite any inner lateral tepal (Fig. 1B). In other cases, the bracteole occupies an almost transverse position; one of the outer tepals is situated between the bract and the bracteole, another is opposite the bracteole, and the third occurs between the inflorescence axis and the bracteole (Fig. 1C).

Japonolirion osense *(Petrosaviaceae)*

Inflorescences are racemose. Flowers are trimerous, actinomorphic, pedicellate, and subtended by a well-developed bract and have a single bracteole; the bract is longer and broader than the bracteole (Fig. 5–7). The bracteole occurs

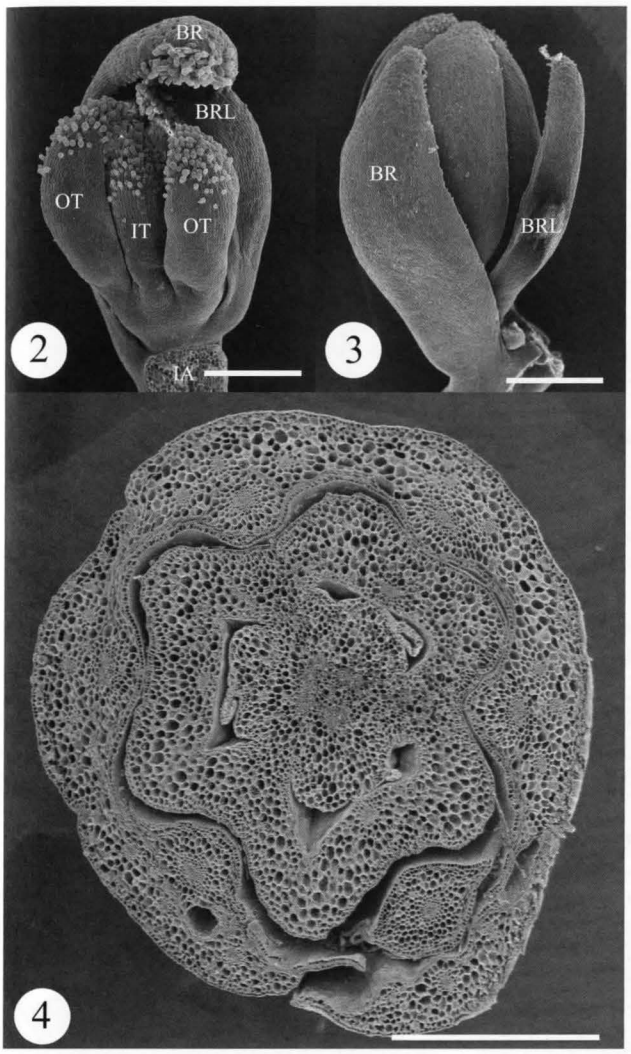

Fig. 2–4.—*Narthecium ossifragum.*—2. Young flower.—3. Young flower, older stage.—4. Transverse section of ovary and perianth at their bases. The tepals and stamen filaments form a short tube at this stage. A free stamen filament is visible at the lower part of the figure. (BR = subtending bract; BRL = bracteole; IA = inflorescence axis; OT = outer tepal; IT = inner tepal. Scale bars: Fig. 2 = 300 μm, Fig. 3 = 600 μm, Fig. 4 = 800 μm.)

at either the left or the right side of the flower, and occupies an intermediate transverse-adaxial or almost transverse position. It is situated opposite an inner lateral tepal. There are six free tepals in two whorls of three, the outer tepals shorter than the inner. In the outer whorl of tepals, one tepal is median adaxial and two are transverse-abaxial if the bracteole is inserted in transverse-adaxial position (Fig. 1B). As in *Narthecium,* bracteole position is unstable, so flower orientation is also unstable (Fig. 1B, C).

Tofieldia *(Tofieldiaceae)*

A common feature of all Tofieldiaceae is the presence of a structure that is usually termed a calyculus (e.g., Engler 1888; Zomlefer 1997; Remizova and Sokoloff 2003). The term "calyculus" can be defined as a group of phyllomes on a pedicel that simulates an outer whorl of the perianth. In terms of homology, the calyculus does not represent part

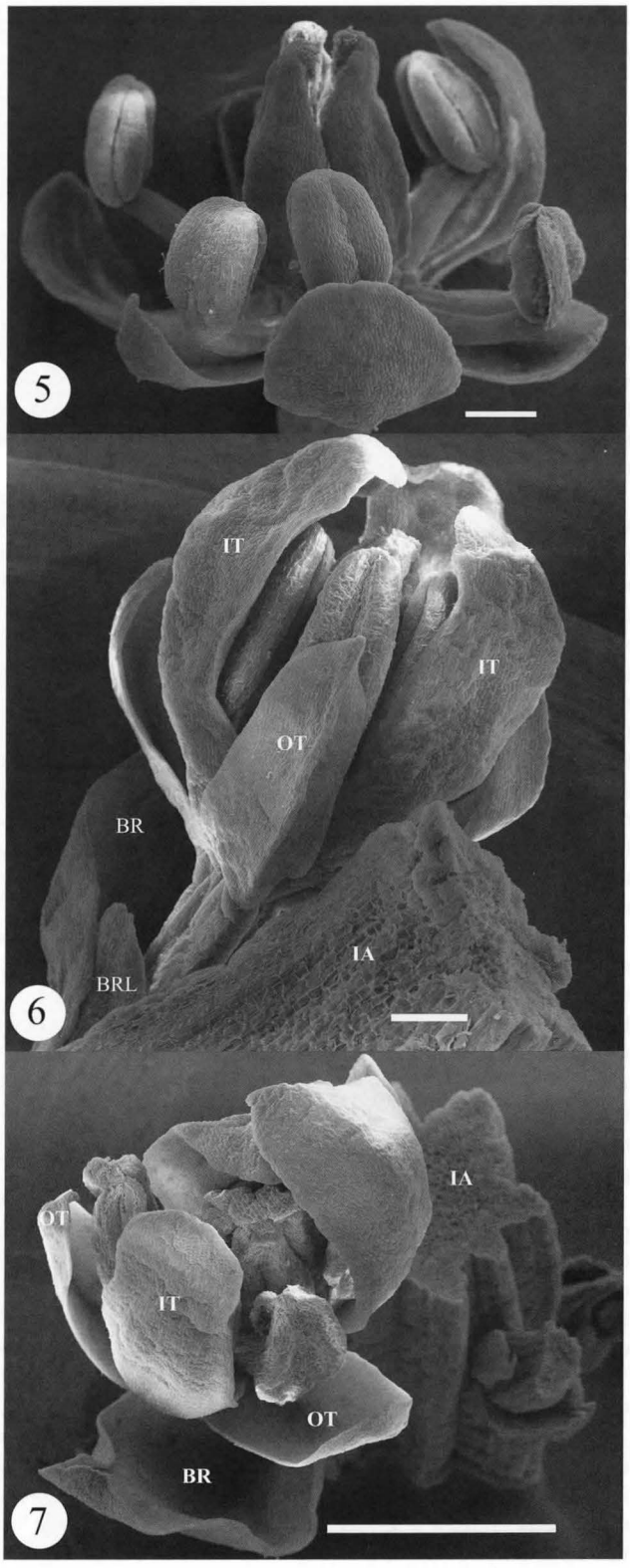

Fig. 5–7.—*Japonolirion osense.*—5. Mature flower.—6. Floral orientation: inflorescence axis dissected.—7. Floral orientation (bracteole not visible). (BR = subtending bract; BRL = bracteole; IA = inflorescence axis; OT = outer tepal; IT = inner tepal. Scale bars: Fig. 5, 6 = 300 μm, Fig. 7 = 1000 μm.)

of the flower. The term "involucre" is sometimes used as a synonym of "calyculus" (e.g., Engler 1888; Zomlefer 1997).

(1) Tofieldia coccinea, T. okuboi *(sect.* Tofieldia*)*

Inflorescences are racemose. Flowers are trimerous, actinomorphic, pedicellate, and subtended by a well-developed bract. Six basally united tepals occur in two distinct whorls. A three-lobed calyculus is inserted on the pedicel just below the flower. The calyculus normally has a radiate structure and consists of three connate scales, one median abaxial, the others transverse adaxial (Fig. 1D), but rarely the fusion of scales is not complete (e.g., the median scale is free). The three scales are of equal size (or sometimes the median scale is shorter than the lateral ones) and alternate with the outer tepals; therefore, the median outer tepal is adaxial.

Early stages of inflorescence and flower development were examined in *T. coccinea,* and later stages in the closely related species *T. okuboi*; these two species are similar in mature floral morphology. Flowers are initiated in an acropetal sequence on the inflorescence axis. A terminal flower is absent, and a residual meristem is visible at the inflorescence apex (Fig. 8). The subtending bract and flower are usually initiated as a common primordium that rapidly divides into two separate primordia by means of a latitudinal slit (Fig. 9); the resulting bract primordium and flower primordium of a given pair may be roughly equal in size, or in some cases the bract primordium is (much) smaller (bract primordia are larger in the lower part of the inflorescence). Occasionally, the subtending bract and the flower primordium are initiated separately and almost simultaneously.

Calyculus initiation is always unidirectional. Lateral scales arise before abaxial one. If floral primordia are not too densely aggregated (especially on the top of the raceme), lateral calyculus scales arise on the adaxial side of the floral meristem as a single crescent-shaped primordium (Fig. 10, 11); each lateral scale forms as an outgrowth of this primordium (Fig. 12). If the floral primordia are densely arranged, each lateral calyculus scale is initiated as a separate primordium (Fig. 13).

The median scale is always initiated later as a separate primordium, either before (in the case of small bract primordia) or after (in the case of large bract primordia) formation of tepals and stamens (Fig. 14). The median scale fuses with two other scales at a later stage of flower development (Fig. 15–19) to form a calyculus tube, by means of "zonal growth" (as, for example, in calyx and stamen tubes of papilionoid legumes: Tucker 1987).

Tepals and stamens are often initiated as common primordia (PA: perianth plus androecium; Fig. 20, 21), although in some cases the tepal and stamen of the same pair arise as separate primordia (Fig. 22, 23). Mode of tepal/stamen initiation is sometimes difficult to determine at early stages of flower development, probably due to the presence of intermediate types.

The PA-primordia of the outer whorl alternate with the primordia of the lateral calyculus scales and the primordium of the subtending bract or the primordium of the median calyculus scale (Fig. 20). Initiation of the inner PA-primordia follows soon after. PA-primordia of the same whorl are initiated simultaneously or reveal unidirectional development, in which case they appear first on the adaxial side of the young flower (Fig. 24, 25). Unidirectional development appears to be correlated with large bract primordium size: the larger the bract primordium, the more prominent the unidirectionality. Since bract primordium size is somewhat variable, the sequence of organ initiation is also relatively unstable. Here we illustrate two of the more common types: (1) The outer median PA-primordium and the inner transverse PA-primordia are initiated first (Fig. 24), followed by the outer transverse PA-primordia and the inner median PA-primordium. (2) All outer PA-primordia are initiated almost simultaneously, followed by the inner transverse PA-primordia and the inner median PA-primordium (Fig. 25). Soon after initiation, each PA-primordium divides into tepal and stamen primordia (Fig. 21). The calyculus scales and tepals grow more rapidly, and soon completely cover the central part of the developing flower.

(2) Tofieldia pusilla *(sect.* Unibracteatae*)*

Inflorescences are racemose. Flowers are trimerous and actinomorphic, with six free tepals in two distinct whorls. The flower-subtending bract is absent, and the calyculus occupies the position of the bract at the base of the pedicel (Fig. 1E, 26, 27). The calyculus of *T. pusilla* has a bilateral structure and consists of three scales, as in members of sect. *Tofieldia.* Normally the lateral scales are fused with the median abaxial one but free from each other (Fig. 26, 27); the median scale is usually longer than the lateral ones. This calyculus structure is atypical for both *Tofieldia* and Tofieldiaceae, in general. The outer tepals alternate with the calyculus scales. The median outer tepal is adaxial. The calyculus of *T. pusilla* is highly variable both in structure and size of scales (Remizova and Sokoloff 2003).

Flowers are initiated in an acropetal sequence along the inflorescence axis as hemispherical bulges (Fig. 28). A terminal flower or flower-like structure is absent. A subtending bract is absent at all stages of development. The first part to be initiated is the calyculus, which arises as a single hemispherical or slightly transversely extended bulge on the abaxial side of the floral primordium (Fig. 29). Soon after initiation, the calyculus becomes horseshoe-shaped. The lateral scales arise as appendages on the common horseshoe-shaped primordium (Fig. 30, 31; Remizova and Sokoloff 2003). In some flowers the lateral scales arise as separate primordia (Fig. 32, 33).

Tepals and stamens are initiated as common PA-primordia or as distinct primordia, but always from the same sector of the floral meristem (Fig. 34–37). Organs of the same whorl arise simultaneously; unidirectional flower development is lacking in *T. pusilla.* Outer PA-primordia alternate with the calyculus scales. Inner PA-primordia are initiated soon after the outer ones. In some cases of distinct tepal and stamen primordial development, the outer stamens and inner tepals are initiated almost simultaneously.

DISCUSSION

In general, floral orientation in lilioid monocots is highly dependent on the presence of a bracteole (Eichler 1875; Engler 1888). In trimerous lateral flowers subtended by a bract, if the bracteole is absent the median tepal of the outer whorl

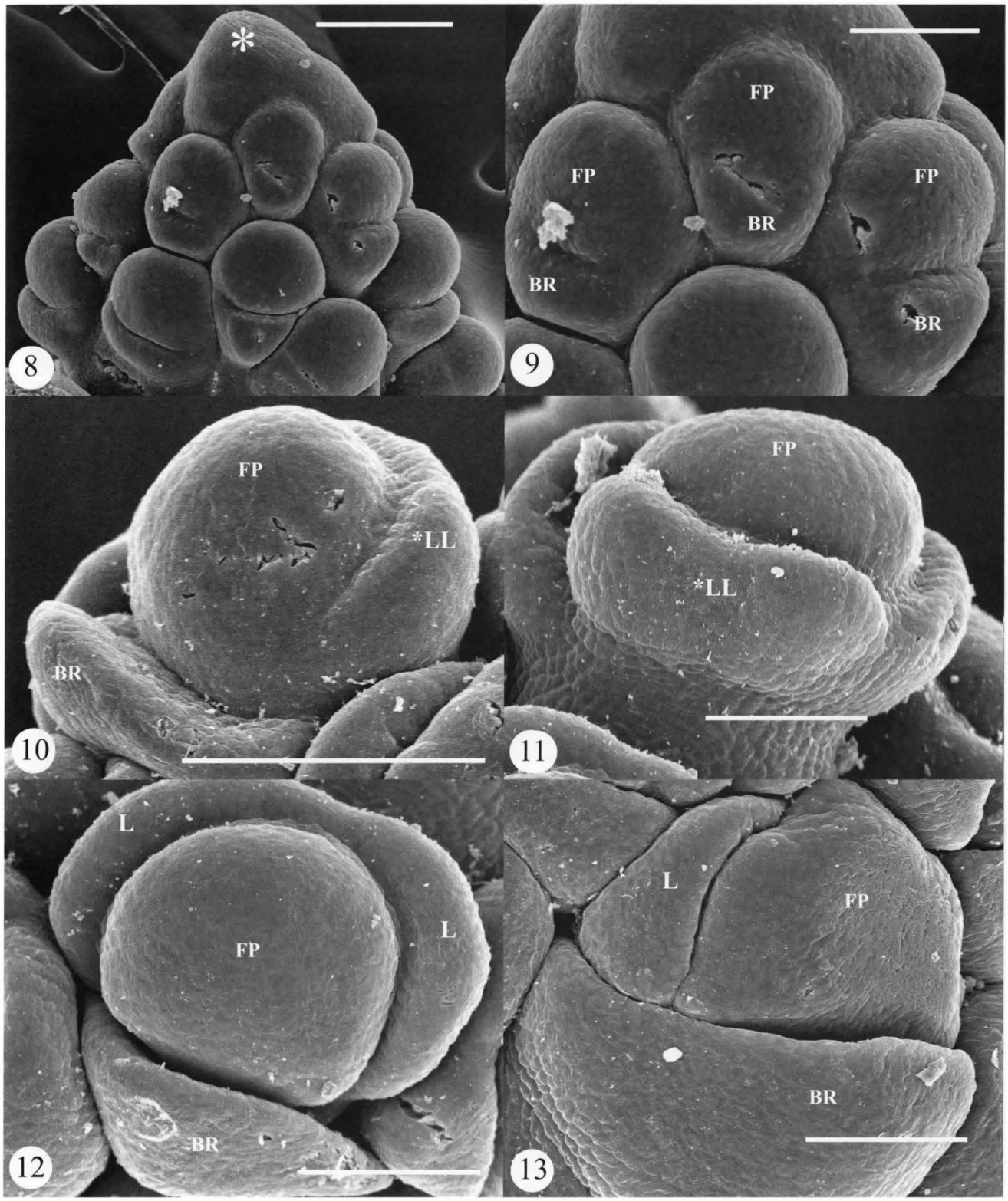

Fig. 8–13.—SEMs of floral ontogenetic stages in *Tofieldia* sect. *Tofieldia* (*T. coccinea*).—8. General view of young inflorescence. Star indicates residual inflorescence meristem.—9. Initiation of subtending bract and flower.—10. Young flower from upper part of inflorescence. Initiation of lateral calyculus scales as common primordium.—11. Young flower from upper part of inflorescence. Initiation of lateral calyculus scales as common primordium. View from adaxial side.—12. Young flower from upper part of inflorescence. Development of lateral calyculus scales.—13. Young flower from lower part of inflorescence. Initiation of lateral calyculus scales as separate primordium. (BR = subtending bract or its primordium; FP = floral primordium; *LL = common primordium of lateral calyculus scales; L = lateral calyculus scale primordium. Scale bars: Fig. 8, 10, 11 = 200 μm, Fig. 9, 12, 13 = 100 μm.)

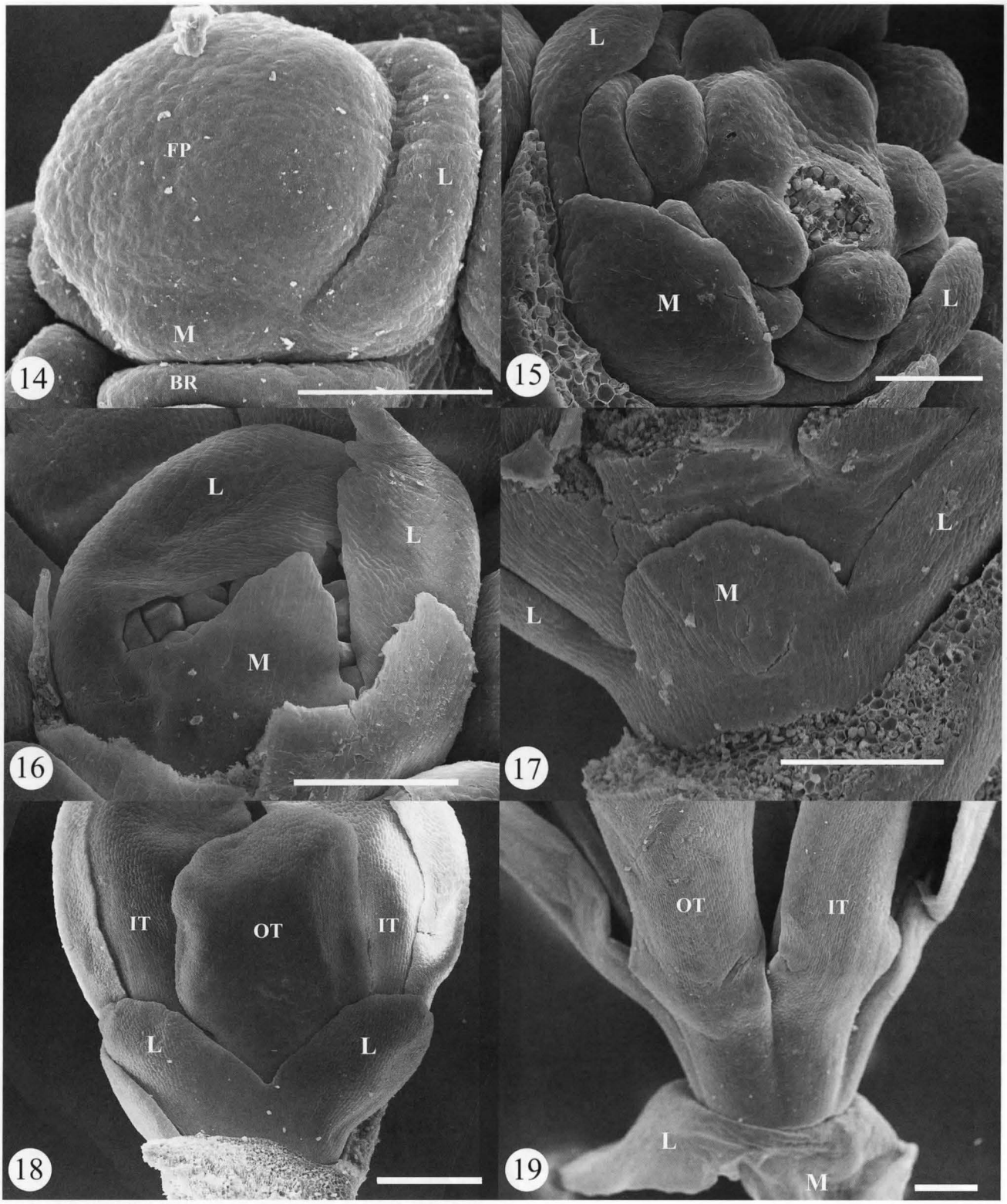

Fig. 14–19.—SEMs of floral ontogenetic stages in *Tofieldia* sect. *Tofieldia* (all *T. coccinea,* except Fig. 18, *T. okuboi*).—14. Development of median calyculus scale (ML). Note already well-developed outer and inner tepals.—15. Fused calyculus scales. Median scale (M) is still smaller than lateral ones (L). Bract removed.—16. Fused calyculus scales. Older stage. Bract partly removed.—17. Fused calyculus scales. Median scale (M) is much smaller than lateral ones (L). Bract removed.—18. Fused calyculus scales. View from adaxial side.—19. Basal part of mature flower. Calyculus is recurved to show floral tube. (BR = subtending bract; FP = floral primordium; L = lateral calyculus scale or its primordium; M = median calyculus scale or its primordium; OT = outer tepal; IT = inner tepal. Scale bars: Fig. 14, 15 = 100 μm, Fig. 16, 18, 19 = 300 μm, Fig. 17 = 200 μm.)

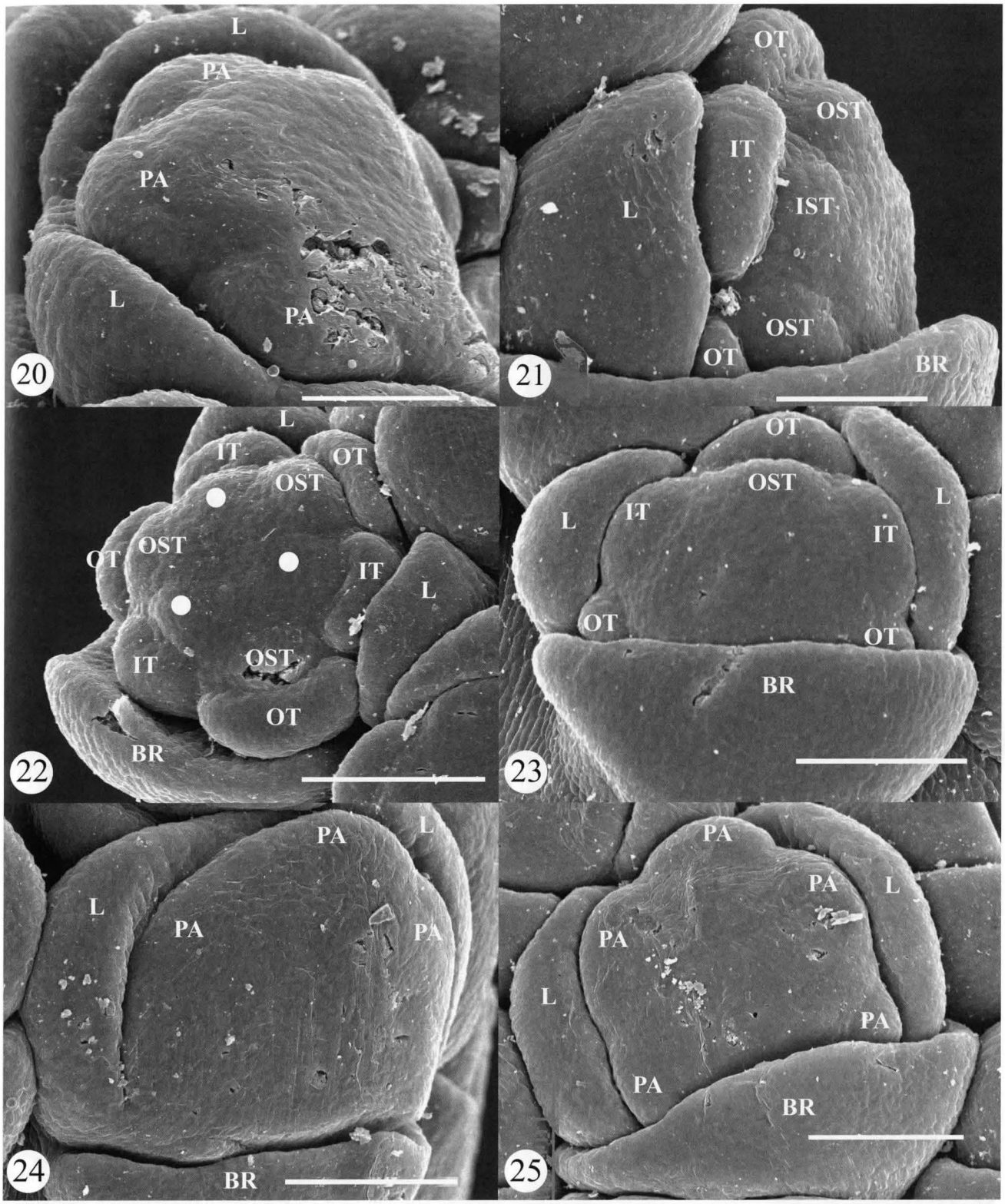

Fig. 20–25.—SEMs of floral ontogenetic stages in *Tofieldia* sect. *Tofieldia* (*T. coccinea*).—20. Simultaneous initiation of PA-primordia.—21. Formation of tepals and stamens from common PA-primordium.—22. Formation of tepals and stamens from separate primordia. Organs of the same whorl are initiated simultaneously. White circles indicate inner stamens.—23. Formation of tepals and stamens from separate primordia. Unidirectional initiation of floral organs.—24–25. Unidirectional initiation of PA-primordia. (BR = subtending bract; L = lateral calyculus scale or its primordium; PA = PA-primordium; OT = outer tepal primordium; IT = inner tepal primordium; OST = outer stamen primordium; IST = inner stamen primordium. Scale bars: Fig. 20 = 80 μm, Fig. 21, 24, 25 = 100 μm, Fig. 22 = 150 μm, Fig. 23 = 120 μm.)

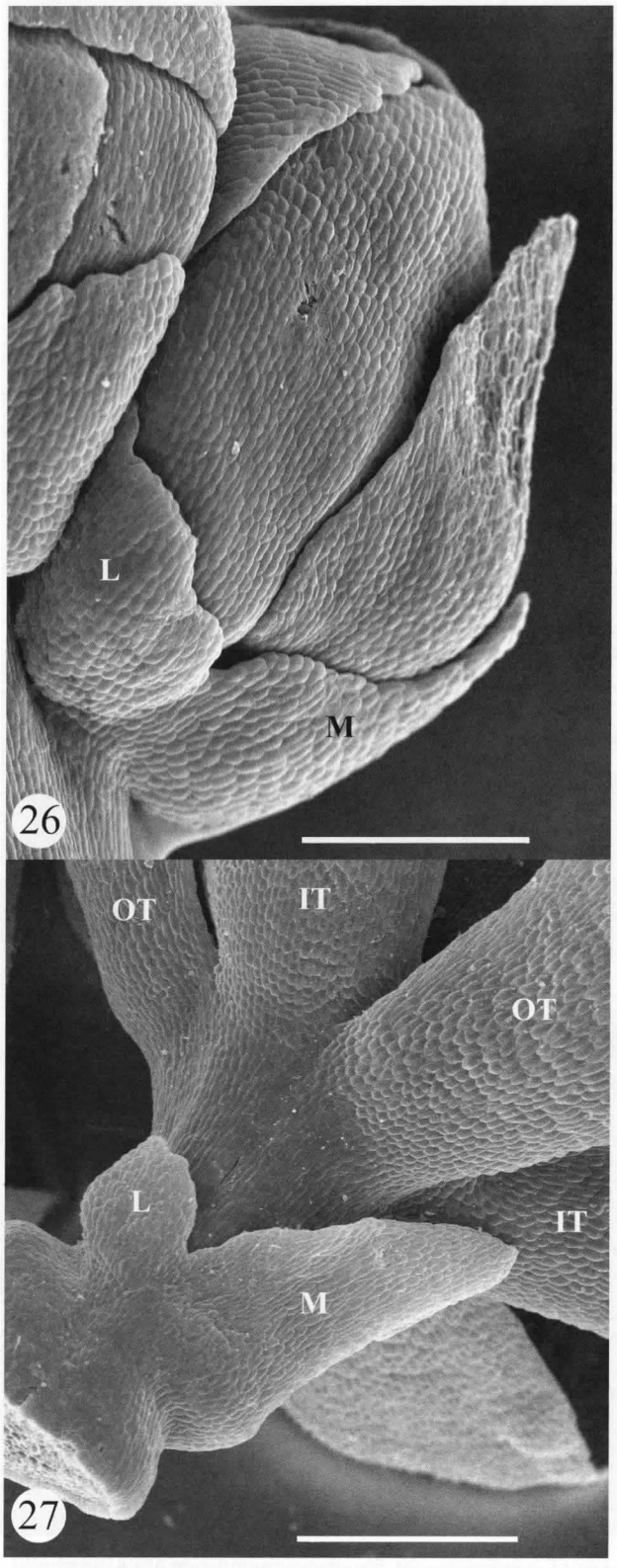

Fig. 26–27.—*Tofieldia* sect. *Unibracteatae* (*T. pusilla*). Mature structure of calyculus.—26. Young sessile flower.—27. Basal part of mature pedicellate flower. (M = median calyculus scale; L = lateral calyculus scale; OT = outer tepal; IT = inner tepal. Scale bars: Fig. 26 = 200 μm, Fig. 27 = 400 μm.)

occupies an abaxial position (see also Rudall and Bateman 2004). This type of floral orientation (Type 1) is most common in lilioid monocots (Fig. 1A). If a bracteole is present, it occurs in either an intermediate (between median adaxial and transverse) or transverse position. The bracteole is always situated in the same radius as one of the inner whorl of tepals. Due to the unstable bracteole position, floral orientation is also unstable (Type 2; Fig. 1B, C). This variability of floral orientation due to various bracteole positions has been described for *Dianella longifolia* R. Br. (Hemerocallidaceae) (Eichler 1875; Engler 1888). Type 3 floral orientation is characteristic of *Tofieldia* and its relatives (Tofieldiaceae), in which the median outer tepal is adaxial due to insertion of a trimerous calyculus below the perianth (Fig. 1D, E).

All three types of floral orientation recognized by Eichler (1875) and Engler (1888) are present in Melanthiaceae s.l., supporting their polyphyletic origin. Type 1 occurs in *Veratrum* L. (Melanthiaceae s.s.; e.g., Endress 1995), and Type 2 in *Japonolirion* and *Narthecium.* The sequence of organ initiation has been studied in detail in two lilioid genera with trimerous flowers and presence of a bracteole: *Allium* L. (Jones and Emsweller 1936) and *Lilium* L. (Greller and Matzke 1970). In both cases, tepal initiation occurs in a spiral direction. The first outer tepal arises opposite the bracteole, and the second between the first outer tepal and bracteole, close to the bracteole. The direction of initiation of the inner tepals is the reverse of the outer. In basal monocots, when the bracteole is absent, the sequence of perianth initiation is either simultaneous in each whorl or unidirectional, but seemingly never spiral (e.g., Endress 1995; Buzgo and Endress 2000; Buzgo 2001; our data). We have not yet observed sequences of perianth development in *Japonolirion* and *Narthecium,* but predict that they have a similar pattern of floral initiation to that of *Allium* and *Lilium.*

Melchior (1964) illustrated a floral diagram of *Petrosavia* with reverse floral orientation to that of *Japonolirion,* with the outer median tepal situated in an abaxial position and a bracteole in the radius of another outer tepal. However, our investigations have demonstrated that the bracteole occurs in the same radius as one of inner tepals in both *Petrosavia* (unpubl. data) and *Japonolirion.* This supports recent analyses of molecular data that place *Japonolirion* as closely related to *Petrosavia* (Cameron et al. 2003). Another feature shared by both *Petrosavia* and *Japonolirion* is that the tepals of the outer whorl are smaller than those of the inner whorl.

Tofieldia has a structure that is unusual for monocots, termed a calyculus, which is common to all Tofieldiaceae. As a consequence, the flower of *Tofieldia* has almost the same orientation as some flowers of Type 2. There are significant differences between the different taxonomic sections of *Tofieldia,* both in calyculus morphology and developmental pattern, but their calyculi are probably homologous (Remizova and Sokoloff 2003). In *Tofieldia* sect. *Tofieldia,* the calyculus is situated just below the flower and has a radial structure (Fig. 1D, 15–19). By contrast, in *T. pusilla* (sect. *Unibracteatae*), a true subtending bract is absent, and the calyculus is inserted at the base of pedicel, and has a bilateral structure (Fig. 1E, 26, 27). The median organ of the calyculus not only occupies the position of the bract but also shows some bract-like features, both in morphology and de-

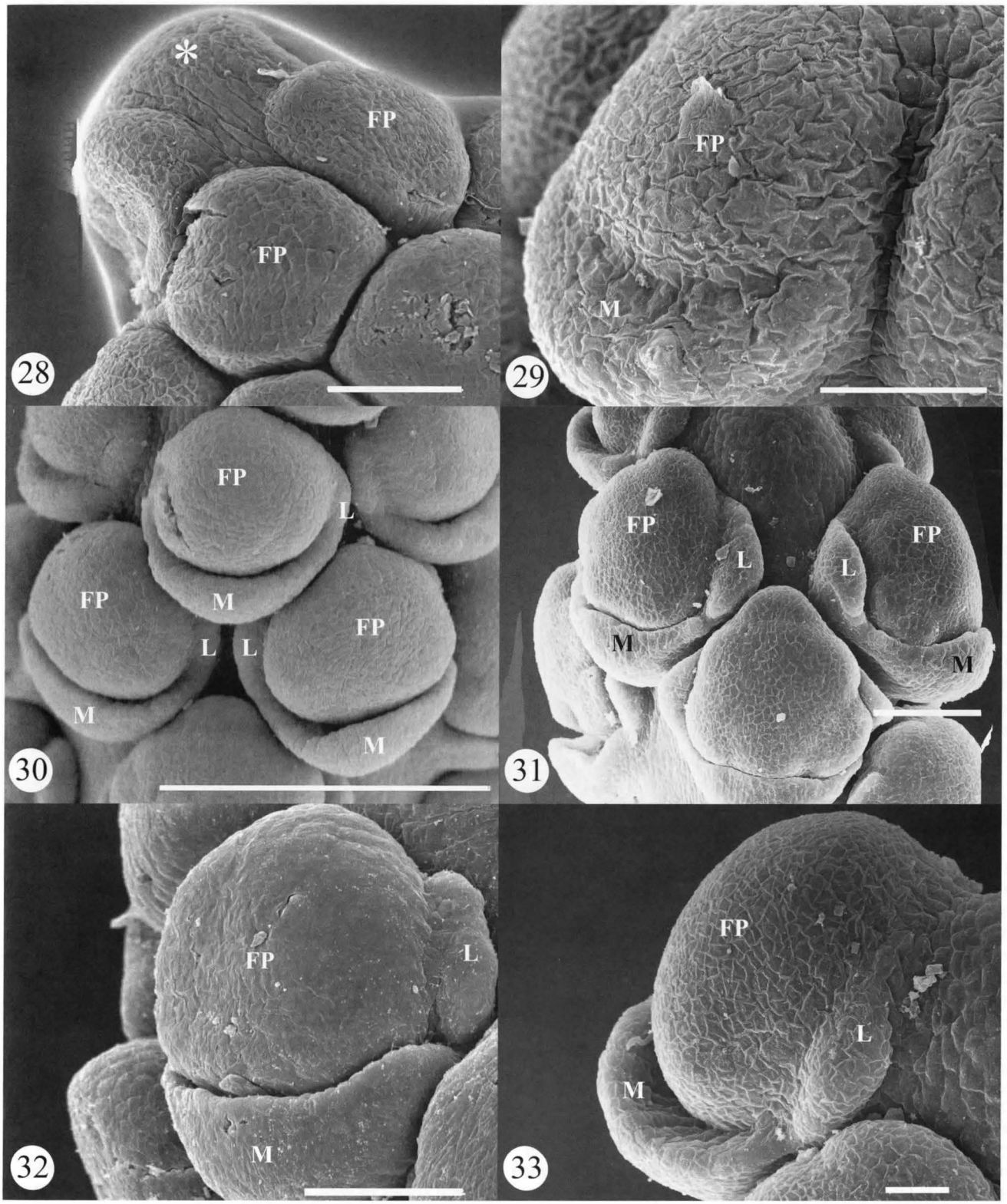

Fig. 28–33.—SEMs of floral ontogenetic stages in *Tofieldia* sect. *Unibracteatae* (*T. pusilla*).—28. Young inflorescence. Hemispherical flower primordia. Star indicates residual inflorescence meristem.—29. Development of calyculus. Median scale is initiated.—30, 31. Development of calyculus. Formation of lateral scales.—32–33. Development of calyculus. Formation of lateral scales as separate primordium. (FP = floral primordium; M = median calyculus scale primordium; L = lateral calyculus scale primordium. Scale bars: Fig. 28, 31 = 100 μm, Fig. 29 = 50 μm, Fig. 30 = 300 μm, Fig. 32 = 80 μm, Fig. 33 = 30 μm.)

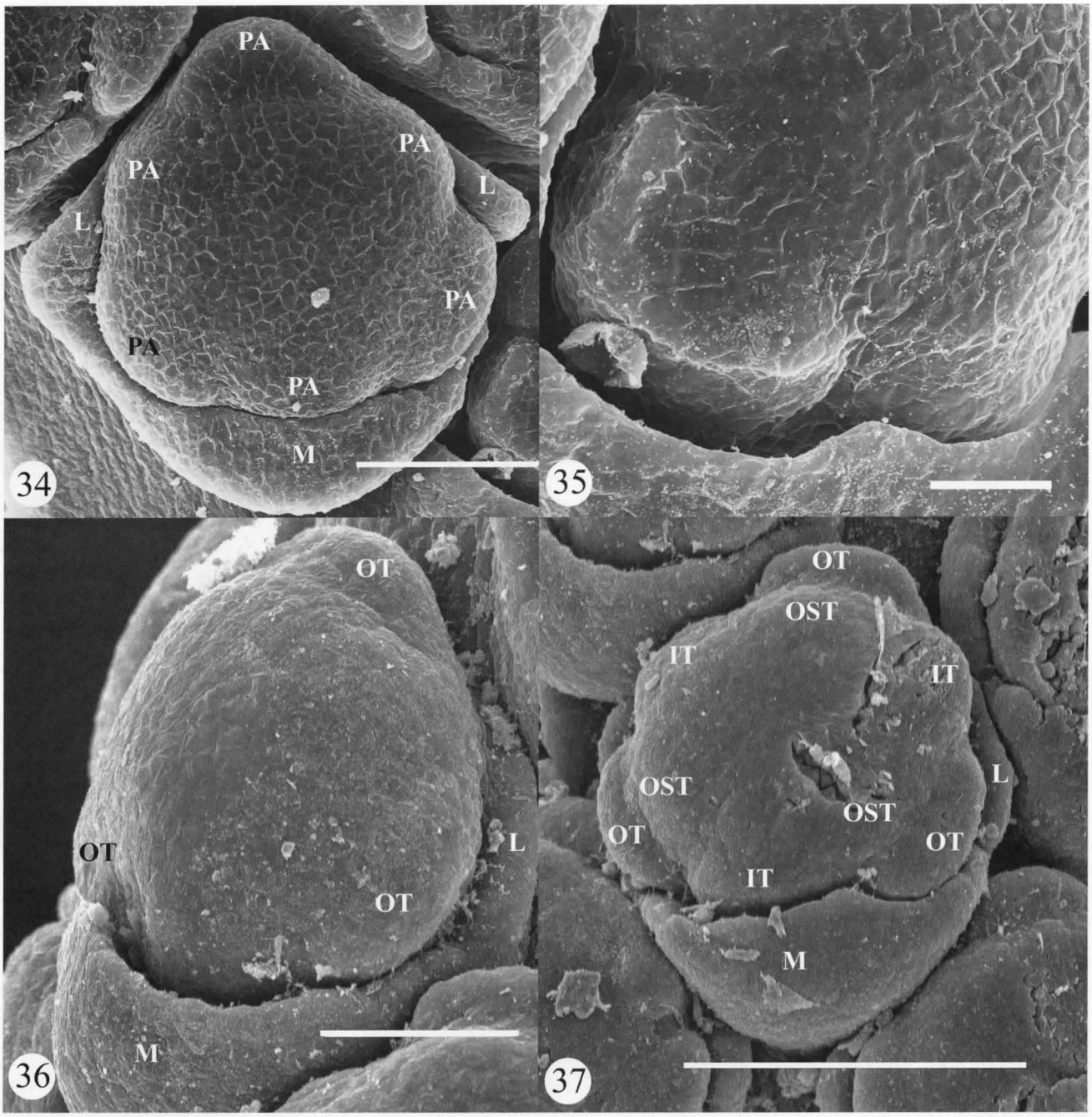

Fig. 34–37.—SEMs of floral ontogenetic stages in *Tofieldia* sect. *Unibracteatae* (*T. pusilla*).—34. Initiation of PA-primordia.—35. PA-primordium of outer whorl.—36–37. Initiation of tepals and stamens as separate primordia. (M = median calyculus scale; L = lateral calyculus scale; PA = PA-primordium; OT = outer tepal primordium; IT = inner tepal primordium; OST = outer stamen primordium. Scale bars: Fig. 34 = 100 μm, Fig. 35 = 30 μm, Fig. 36 = 80 μm, Fig. 37 = 200 μm.)

velopment (Remizova and Sokoloff 2003). In *T. coccinea* (sect. *Tofieldia*) the calyculus often develops as two separate primordia: the common primordium of the lateral scales, and the primordium of the median scale. This pattern of lateral calyculus scale initiation in *T. coccinea* resembles prophyll initiation in the vegetative bud. However, the lateral scale cannot be interpreted as a prophyll because in basal monocots with racemose inflorescences prophylls in the floral region (i.e., bracteoles), if present, are not adaxial. Moreover, the modification of prophyll development in sect. *Unibracteatae* (*T. pusilla*) would be unclear if we interpreted the lateral scales of sect. *Tofieldia* as a two-keeled prophyll. Another possibility is that the two lateral calyculus scales represent two lateral prophylls. We consider such an interpretation unlikely, because of the presence of a third (abaxial) calyculus scale, which should be interpreted as the next phyllome on the lateral shoot. In other monocots, we do not know any example of fusion between lateral prophylls and the next phyllome on the lateral shoot.

Formation of "hybrid" organs that combine characters of

the flower-subtending bract and the first median abaxial phyllome on the pedicel is a feature common to *T. pusilla* and some other Alismatales, such as Aponogetonaceae, Juncaginaceae and *Potamogeton densus* L. (Potamogetonaceae), in addition to the putatively basal monocot, *Acorus* (Posluszny and Sattler 1973; Buzgo and Endress 2000; Buzgo 2001; Remizova and Sokoloff 2003). Formation of similar bract-like organs may be regarded as additional morphological evidence for a close relationship between these taxa. However, this similarity apparently represents a homoplastic tendency (or even a possible symplesiomorphy) rather than a synapomorphy. In *Tofieldia pusilla,* the first abaxial phyllome exhibiting bract-like features is the median calyculus scale, whereas in Acoraceae (Fig. 1G), Aponogetonaceae, Juncaginaceae, and *Potamogeton* L. this is the outer median tepal.

According to Buzgo (2001), in Alismatales s.l., unidirectional development is often correlated with structures resembling subtending bracts (and absence of a true bract), and with formation of peloria (or more precisely, terminal flower-like structures: Buzgo et al. 2004). Our data show no such correlation in *Tofieldia.* Among species examined here, unidirectional floral development occurs in *Tofieldia coccinea* (sect. *Tofieldia*), but this species has a true subtending bract and no terminal flower-like structures. In *T. pusilla* the true bract is absent, being replaced by the median organ of the bract-like calyculus, but organ initiation is simultaneous in each whorl.

There is some similarity between calyculus development in *Tofieldia* sect. *Tofieldia* and tepal development in some lilioid monocots. In *T. coccinea* the median abaxial calyculus scale is considerably delayed in development. In *Veratrum* (Melanthiaceae s.s., Liliales) and *Bulbine* Wolf (Asphodelaceae, Asparagales) there is no calyculus or bracteole on the pedicel, but the development of the median outer tepal is delayed (Endress 1995). Thus, unidirectional development is probably a result of the presence of a closely situated large bract primordium in these cases (see also Endress 1999).

Our data show that the presence or absence of a calyculus and bracteole are stable characters within relatively large taxonomic groups of basal monocots. The presence of a bracteole and calyculus seems to be more stable than the presence of a bract, since a bract may be either present or absent in both *Tofieldia* and *Potamogeton.* Characters such as presence of bracteoles and calyculus can be used as taxonomic characters at the family level, but are homoplastic at higher taxonomic levels. For example, bracteoles are similar in members of Liliaceae, Nartheciaceae, and Petrosaviaceae, which are not closely related. The calyculus of Tofieldiaceae can be compared to a whorl of leaves below the flower in *Paris* L. and *Trillium* L., which belong to the relatively distantly related family Melanthiaceae s.s. (Liliales). The calyculus can also be compared to (pseudo)whorls of bracts in Alismataceae and the "spathe" of Hydrocharitaceae (Remizova and Sokoloff 2003). These two families are more closely related to Tofieldiaceae than Melanthiaceae (Chase et al. 2000; APG II 2003), but there is currently no direct evidence that these organs represent a synapomorphy for these families.

CONCLUSIONS

Among basal monocots, floral orientation is often a conservative feature in groups of family rank such as Acoraceae (Buzgo and Endress 2000), Araceae (Buzgo 2001), Tofieldiaceae (Remizova and Sokoloff 2003), and many others. Interestingly, Araceae and Tofieldiaceae, which belong in the same clade, share a similar type of floral orientation that is otherwise rare in monocots (Fig. 1). When a bracteole is present and its position on the pedicel is unstable, floral orientation is also unstable, often within a given inflorescence. In general, the orientation of lateral flowers in monocots is highly dependent on the presence and position of additional phyllomes on the pedicel. In *Japonolirion, Lilium, Narthecium,* and many others, the additional phyllome is represented by a bracteole. During flower development, an outer whorl tepal is never initiated in the radius of the bracteole; an inner whorl tepal is initiated there instead. In *Tofieldia,* the additional phyllomes are represented by calyculus scales and the outer whorl tepals are initiated alternating with the calyculus scales. Thus, the type of floral orientation that occurs in *Tofieldia* and other Tofieldiaceae is unusual for monocots.

Our investigation reveals striking differences in pattern of calyculus development within *Tofieldia* between members of sect. *Tofieldia* and sect. *Unibracteatae*: phyllome primordia surround the floral primordium in a very different manner, as flower subtending bract and lateral scale primordia in *T. coccinea,* and as a calyculus primordium in *T. pusilla.* The difference between the two sections is especially clear in position of lateral calyculus scales. In sect. *Tofieldia* the scales are initiated in transverse-adaxial position, but in almost transverse position in sect. *Unibracteatae.* Nevertheless, in both *T. coccinea* and *T. pusilla* the type of floral orientation is relatively stable (i.e., the median outer tepal is adaxial). Thus, it is possible that floral orientation in *Tofieldia* represents more than the result of the presence of a calyculus.

As might be expected in view of their polyphyletic origin, there are significant differences in inflorescence and flower morphology, floral orientation and flower development between members of the species examined here. Data on flower and inflorescence morphology and development are important for investigations of the systematics and phylogeny of basal monocots, even if many of the similarities represent homoplastic tendencies or symplesiomorphies rather than true synapomorphies.

ACKNOWLEDGMENTS

We are grateful to Matyas Buzgo, Vladimir Choob, Peter Endress, Claudia Erbar, Peter Leins, Evgeny Mavrodiev, Rolf Rutishauser, and Wendy Zomlefer for helpful discussion and comments. Several people kindly collected material of *Tofieldia,* including N. Bocharova, E. Bubnova, E. Ivashkin, K. Kondo, V. Kostina, L. Moskvicheva, M. Pletz, E. Pospelova, and P. Zhmylev. Collection of material of *Tofieldia okuboi* by P. Zhmylev and K. Kondo was made possible by support from a grant-in-aid for Scientific Research Program (A)(1) # 1425501400. A. S. Koryakin (Deputy Director of Kandalaksha State Nature Reserve) kindly helped with organizing collection of material. M. R. and D. S. gratefully

acknowledge funding from the Russian Foundation for Basic Research (Project # 03-04-48427), a President of Russian Federation Grant for leading scientific schools (No. 1898.2003.4), and the Programme "Universities of Russia" (No. 07.03.075).

LITERATURE CITED

AMBROSE, J. D. 1980. A re-evaluation of the Melanthioideae (Liliaceae) using numerical analyses, pp. 65–81. *In* C. D. Brickell, D. F. Cutler, and M. Gregory [eds.], Petaloid monocotyledons: horticultural and botanical research. Academic Press, London, UK.

ANGIOSPERM PHYLOGENY GROUP [APG]. 1998. An ordinal classification for the families of flowering plants. *Ann. Missouri Bot. Gard.* **85**: 531–553.

ANGIOSPERM PHYLOGENY GROUP [APG II]. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Bot. J. Linn. Soc.* **141:** 399–436.

BUZGO, M. 2001. Flower structure and development of Araceae compared with alismatids and Acoraceae. *Bot. J. Linn. Soc.* **136**: 393–425.

———, AND P. K. ENDRESS. 2000. Floral structure and development of Acoraceae and its systematic relationships with basal angiosperms. *Int. J. Pl. Sci.* **161**: 23–41.

———, D. E. SOLTIS, P. S. SOLTIS, AND H. MA. 2004. Towards a comprehensive integration of morphological and genetic studies of floral development. *Trends Pl. Sci.* **9**: 164–173.

———, ———, ———, S. KIM, H. MA, B. A. HAUSER, J. LEEBENS-MACK, AND B. JOHANSEN. 2006. Perianth development in the basal monocot *Triglochin maritima* (Juncaginaceae), pp. 107-125. *In* J. T. Columbus, E. A. Friar, J. M. Porter, L. M. Prince, and M. G. Simpson [eds.], Monocots: comparative biology and evolution (excluding Poales). Rancho Santa Ana Botanic Garden, Claremont, California, USA.

CAMERON, K. M., M. W. CHASE, AND P. J. RUDALL. 2003. Recircumscription of the monocotyledonous family Petrosaviaceae to include *Japonolirion*. *Brittonia* **55**: 214–225.

CHASE, M. W. 2003. Monocot relationships: evidence from all three genomic compartments. Third international conference on the comparative biology of the monocotyledons. Rancho Santa Ana Botanic Garden, Claremont, California, USA, 31 Mar–4 Apr 2003. Abstract.

———, D. E. SOLTIS, P. S. SOLTIS, P. J. RUDALL, M. F. FAY, W. H. HAHN, S. SULLIVAN, J. JOSEPH, T. J. GIVINISH, K. J. SYTSMA, AND J. C. PIRES. 2000. Higher-level systematics of the monocotyledons: an assessment of current knowledge and a new classification, pp. 3–16. *In* K. L. Wilson and D. A. Morrison [eds.], Monocots: systematics and evolution. CSIRO Publishing, Collingwood, Victoria, Australia.

———, D. W. STEVENSON, P. WILKIN, AND P. J. RUDALL. 1995. Monocot systematics: a combined analysis, pp. 685–730. *In* P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries [eds.], Monocotyledons: systematics and evolution. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

CRUDEN, R. W. 1991. A revision of *Isidrogalvia* (Liliaceae): recognition of Ruiz and Pavón's genus. *Syst. Bot.* **16**: 270–282.

DAHLGREN, R. M., H. T. CLIFFORD, AND P. F. YEO. 1985. The families of the monocotyledons. Springer-Verlag, Berlin, Germany. 520 p.

DAUMANN, E. 1970. Das Blütennektarium der Monocotyledonen unter besonderer Berücksichtigung seiner systematischen und phylogenetischen Bedeutung. *Feddes Repert.* **80**: 463–590.

EICHLER, A. W. 1875. Blüthendiagramme. W. Engelmann, Leipzig, Germany. 347 p.

EIE, S. 1972. Floral anatomy in *Tofieldia pusilla* (Michx.) Pers. with special reference to the gynoecium. *Norweg. J. Bot.* **19**: 31–36.

EL-HAMIDI, A. 1952. Vergleichend-morphologische Untersuchungen am Gynoeceum der Unterfamilien Melanthioideae und Asphodeloideae der Liliaceae. *Arbeiten Inst. Allg. Bot. Univ. Zürich* **4**: 1–50.

ENDRESS, P. K. 1995. Major traits of monocot flowers, pp. 43–79. *In* P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries [eds.], Monocotyledons: systematics and evolution. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

———. 1999. Symmetry in flowers: diversity and evolution. *Int. J. Pl. Sci.* **160**: S3–S23.

ENGLER, A. 1888. Liliaceae, pp. 10–91. *In* A. Engler und K. Prantl [eds.], Die natürlichen Planzenfamilien. W. Engelmann, Leipzig, Germany.

GRELLER, A. M., AND E. B. MATZKE. 1970. Organogenesis, aestivation, and anthesis in the flower of *Lilium tigrinum*. *Bot. Gaz.* **131**: 304–311.

IGERSHEIM, A., M. BUZGO, AND P. K. ENDRESS. 2001. Gynoecium diversity and systematics in basal monocots. *Bot. J. Linn. Soc.* **136**: 1–6.

JONES, H., AND S. EMSWELLER. 1936. Development of the flower and macrogametophyte of *Allium cepa*. *Hilgardia* **10**: 415–428.

LEINFELLNER, W. 1963. Über die Variabilität der Blüten von *Tofieldia calyculata*. III. Zusammenfassende Übersicht der vorgefundenen Abweichungen. *Oesterr. Bot. Z.* **110**: 395–430.

MELCHIOR, H. 1964. Engler's Syllabus der Pflanzenfamilien. II. Bornträger, Berlin, Germany. 666 p.

POSLUSZNY, U., AND R. SATTLER. 1973. Floral development of *Potamogeton densus*. *Canad. J. Bot.* **51**: 647–656.

REMIZOVA, M. V., AND D. D. SOKOLOFF. 2003. Inflorescence and floral morphology in *Tofieldia* (Tofieldiaceae) compared with Araceae, Acoraceae and Alismatales s. str. *Bot. Jahrb. Syst.* **124**: 255–271.

RUDALL, P. J. 2002. Homologies of inferior ovaries and septal nectaries in monocotyledons. *Int. J. Pl. Sci.* **163**: 261–276.

———, AND R. M. BATEMAN. 2004. Evolution of zygomorphy in monocot flowers: iterative patterns and developmental constraints. *New Phytol.* **162**: 25–44.

STERLING, C. 1979. Comparative morphology in the Liliaceae: Tofieldieae. *Bot. J. Linn. Soc.* **79**: 321–332.

STEVENSON, D. W., AND H. LOCONTE. 1995. Cladistic analysis of monocot families, pp. 543–578. *In* P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries [eds.], Monocotyledons: systematics and evolution. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

TAKHTAJAN, A. L. 1987. Systema Magnoliophytorum (Sistema magnoliofitov). Nauka, Leningrad, USSR. 439 p. [in Russian.]

———. 1994. New families of the monocotyledons. *Bot. Zhurn.* **79**: 65–66.

———. 1997. Diversity and classification of flowering plants. Columbia University Press, New York, USA. 663 p.

TAMURA, M. N. 1998. Nartheciaceae. *In* K. Kubitzki [ed.], The families and genera of vascular plants, Vol. 3. Monocotyledons, Lilianae (except Orchidaceae). Springer-Verlag, Berlin, Germany.

THORNE, R. F. 1992. Classification and geography of the flowering plants. *Bot. Rev.* 58: 225–348.

TUCKER, S. C. 1987. Floral initiation and development in legumes, pp. 183–239. *In* C. H. Stirton [ed.], Advances in legume systematics, pt. 3. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

UTECH, F. N. 1978*a*. Comparison of the vascular floral anatomy of *Xerophyllum asphodeloides* (L.) Nutt. and *X. tenax* (Pursh) Nutt. (Liliaceae–Melanthioideae). *Ann. Carnegie Mus.* **47**: 147–167.

———. 1978*b*. Floral vascular anatomy of *Pleea tenuifolia* Michx. (Liliaceae–Tofieldiae) and its reassignment to *Tofieldia*. *Ann. Carnegie Mus.* **47**: 423–453.

———. 1978*c*. Floral vascular anatomy of the monotypic Japanese *Metanarthecium luteoviride* Maxim. (Liliaceae–Melanthioideae). *Ann. Carnegie Mus.* **47**: 455–477.

———. 1978*d*. Vascular floral anatomy of the East Asian *Helonias*

bullata L. (Liliaceae–Helonieae), with a comparison to the Asian *Heloniopsis orientalis*. *Ann. Carnegie Mus.* **47**: 169–191.

———. 1984. Floral vascular anatomy of *Japonolirion osense* Nakai (Liliaceae) and its tribal relationship. *Ann. Carnegie Mus.* **53**: 447–461.

———, AND S. KAWANO. 1981. Vascular anatomy of the East Asian *Heloniopsis orientalis* (Thunb.) C. Tanaka (Liliaceae–Helonieae). *Bot. Mag. (Tokyo)* **94**: 295–311.

VAN HEEL, W. A. 1988. On the development of some gynoecia with septal nectaries. *Blumea* **36**: 477–504.

ZOMLEFER, W. B. 1997. The genera of Tofieldiaceae in southeastern United States. *Harvard Pap. Bot.* **2**: 179–194.